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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSE OF *ATRIPLEX CANESCENS* (PURSH) NUTT UNDER METALLIC STRESS

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Abstract

The pollution of the environment and soils by heavy metals is one of the major problems of our time. Our aim is to study the accumulator effect of heavy metals (lead and cadmium) by plant *Atriplex canescens* stressed after 60 days of sowing. Five different doses were applied to *Atriplex canescens* for two weeks: Pb and Cd (0, 2500, 5000, 7500, and 10000 ppm). Physiological and biochemical analyses showed a decrease in foliar chlorophyll pigment content (chlorophyll a, b and total) and water content as a function of the increasing concentration of heavy metals at leaf levels.

The chlorophyll b content is much higher than that of chlorophyll a, which represents a 50% increase in chlorophyll b content compared to chlorophyll a. The lowest water contents in leaves (29% and 20.01%) are obtained at a dose of 10000 ppm (lead, cadmium) respectively. The results show that the tolerance to metal stress is largely due to the degree of plant development.

Key words : Metallic stress, *Atriplex canescens* (Pursh) Nutt, chlorophyll content, relative water content.

Introduction

Environmental pollution has become a real problem threatening our ecosystems with detrimental effects on vegetable production. Among the main pollutants are heavy metals, persistent, which accumulate in the soil by contaminating the environment. All the ETM are potentially toxic for plants according to their concentration in an environment and to their essential character or not for the plant (Lotmani and Mesnoua, 2011). The trace elements selected for this work are among metallic pollutants toxin most frequently met in soils: the cadmium for its phytodisponibilité and its high toxicity and the lead, considered as little mobilizable but also very toxic when assimilated by plants.

In stressful conditions, plants can react by implementing mechanisms, among others, physiological (Martinez *et al.,* 2007) and biochemical (Brugnoli and Lauteri, 1991 ; Attia, 2007).

The interest shown in the biochemical characteristics of adaptation to environmental constraints required us to study the accumulation of chlorophyll pigments, often used as a diagnostic tool for diagnosing the functional state of photosystems under metallic stress conditions.

The relative water content corresponds to a direct physiological significance of the hydric state of the vegetable (Collinson *et al.,* 1997). It is an indicator physiological parameter of the resistance of species to water stress. Plant species that maintain relative high-water foliar levels are considered to be drought-resistant species.

Several species belonging to the genus *Atriplex* are well adapted to harsh environmental conditions and is characterized by its great diversity with more than 400 species (Le Houérou, 1992). They are a useful material for the identification of physiological mechanisms and genes involved in resistance to abiotic stresses (Wang and Showalter, 2004). The *Atriplex canescens* (Pursh) Nutt is a halophyte of the Chenopodiaceae family, from North America (Mulas and Mulas, 2004).

In this context, our research work aims to evaluate the effect of heavy metals lead and cadmium on the physiological and biochemical parameters of the *Atriplex canescens*.

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Materials and Methods

Plant material

The plant material included in this study concerns seeds of the *Atriplex canescens* (Pursh) Nutt of the chenopodoaceae family. It was chosen because of its use in the laboratory as a model plant for its resistance and adaptation to abiotic stresses.

These seeds are peeled manually, disinfected with bleach for 5 minutes and then rinsed thoroughly with distilled water to remove all traces of chlorine, they have been dried before germination.

Methods of culture

The Seeds are germinated in alveoli filled with compost up to the seedling stage in a greenhouse. Then, seedlings are transplanted in cylinders (height 50 cm and diameter 20 cm), completely papered with a coat of gravels to assure the drainage, filled with a mixture sand /compost (2V/V). A watering every three days is operated to the nutrient solution of Hoagland (1938) to 30 % of the capacity of retention of the substrate (Khedim *et al.,* 2017).

Application of stress

Metallic stress was applied to the plant after 60 days of culture for two weeks. Five metal doses (0, 2500, 5000, 5000, 7500 and 10000 ppm) were applied for lead and cadmium with three repetitions for all metal doses applied**.**

After two weeks of the stress, plants are taken, leaves, and roots separated, and dried for 24 hours at 80°C. Then, the dry samples are crushed is deposited in closed vials using plasma plug.

Parameters analysed

Determination of chlorophyll

The extraction of chlorophyll a and b is carried out according to the method of Lichtenthaler (1987) and Shabala et *al., (1*998).

Chlorophyll "a", chlorophyll "b" concentrations are performed using a UV spectrophotometer at the respective optical densities of 662 nm and 664 nm.

The contents of chlorophyll a, b and totals are calculated by the following formulas:

Chl a = 9.784 x do (662)-0.99 × Do (664)

Chl b = 21.42 x Do (664)-4.65 × Do (662)

Total Chlorophyll = Chl a + Chl b.

Relative water content (RWC)

The relative water content is determined by the method of Barrs and Weatherley (1962) and then by Scippa *et al.,* (2004).

The relative RWC water content is calculated according to the following formula:

RWC (%) = [(FW – DW)/(FTW – DW)] × 100

FW: Fresh Weight (g)

DW: Dry Weight (g)

FTW: Full Turgor weight (g)

Statistical analysis

The results obtained are processed statistically using the Statbox software. Version 6.4, of a variance analysis using the test of Newman-Keuls P = 5%.

Results

Chlorophyll content

Chlorophyll a, b, and total content of the leaves of the *Atriplex canescens* stressed by lead

The results showed a significant decrease in chlorophyll a, b and total content in leaves of plants subjected to lead stress compared to the control plant (Fig. 1). The chlorophyll b content is much higher than that of chlorophyll a, which represents an increase of 50% in chlorophyll b content than that recorded in chlorophyll a.

Statistical analysis shows that the accumulation of chlorophyll a and total is significant in the leaves with all the lead treatments. However, chlorophyll b is not significant.

Content of chlorophyll a, b and total of the leaves of the *Atriplex canescens* stressed by cadmium

The results showed a significant decrease in chlorophyll a, b and total content in the leaves of plants subjected to cadmium stress relative to the control plant (Fig. 2). Chlorophyll a is much lower than that of chlorophyll b and total.

The chlorophyll a content in control plants was 1.40 mg. g^{-1} fresh weight, this content is higher than that obtained for plants stressed by cadmium.

The Application of cadmium at doses 2500, 5000, 7500, and 10000 ppm results in a decrease in chlorophyll b content (2.51, 2.43, 2.28, and 2.01 mg. g^{-1} fresh weight) successively, compared to the control plant (2.61 mg. g^{-1} fresh weight).

The total chlorophyll content recorded in control plants is much higher; it is 4.02 mg. g^{-1} fresh weight. The enrichment of the solutions at 2500, 5000, 7500, and 10000 ppm of cadmium resulted in a decrease in total chlorophyll content (3.91, 3.71, 3.49, and 3.20 mg. g^{-1} fresh weight, respectively).

Statistical analysis showed a significant effect on the

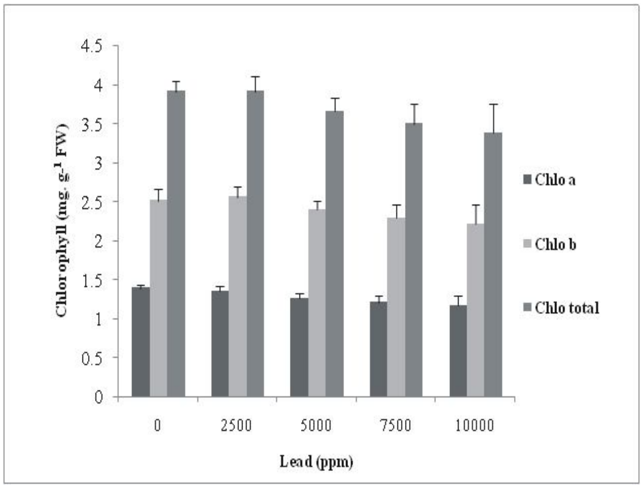

Fig. 1: Chlorophyll **a, b** and **total** content (mg. g^{-1} fresh weight) of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed by lead.

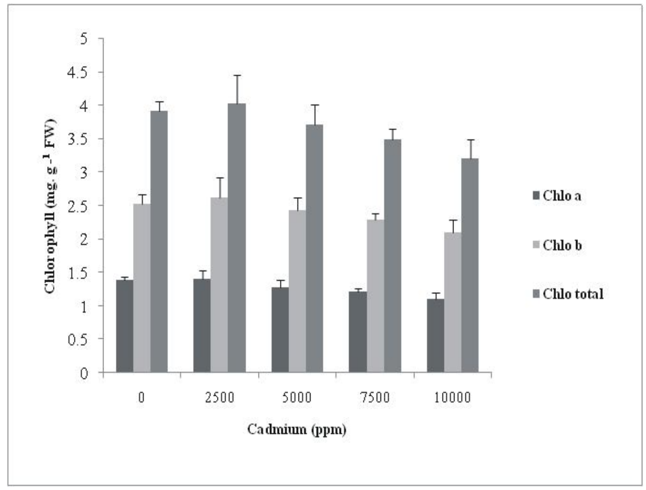

Fig. 2: Chlorophyll **a, b** and **total** content (mg. g^{-1} fresh weight) of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed by cadmium.

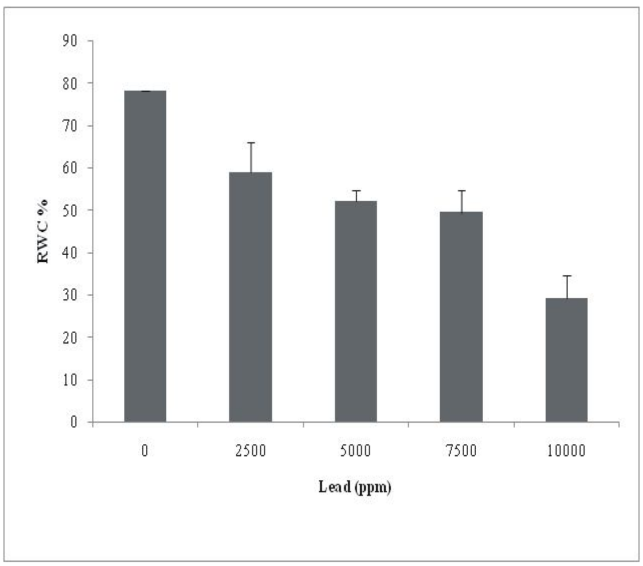

Fig. 3: Relative water content of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed by lead.

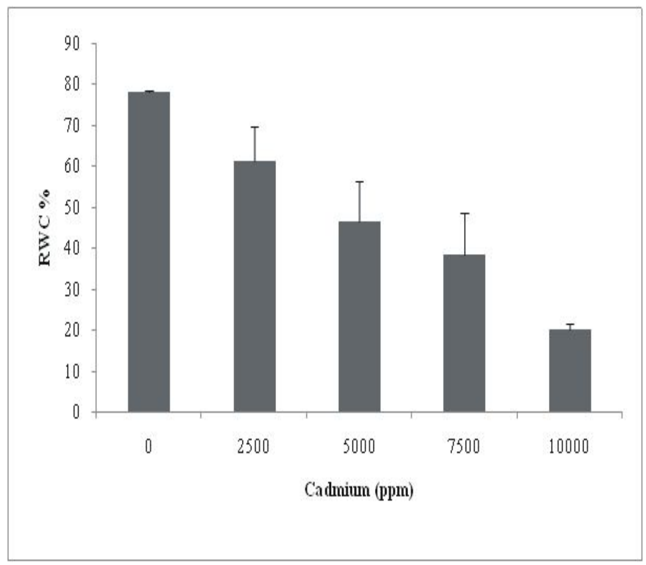

Fig. 4: Relative water content of the leaves of the *Atriplex canescens* (Pursh) Nutt stressed by cadmium.

accumulation of chlorophyll a, b and total in the leaves of the plant for all applied doses of cadmium.

Relative water content

Relative water content of the leaves of the *Atriplex canescens* stressed by lead

The results obtained indicate that the lead stress applied to the plant leads to a decrease in the water content in the leaves at 2500, 5000, 7500 and 10000 ppm of lead (58.93, 52.17, 49.46and 29.22%, respectively) compared to the control (78.18%) (Fig. 3).

Analysis of the variance reveals a very highly significant difference in the leaves of the plants stressed compared to the leaves of the control plants.

Relative water content of the leaves of the *Atriplex canescens* stressed by cadmium

The results found for cadmium stressed plants at different concentrations (2500, 5000, 7500, and 10000 ppm) indicate a decrease in water content in the leaves of the *Atriplex canescens* (Fig. 4).

The water content obtained was lower in the leaves of the plant (61.18, 46.50, 38.32 and 20.01%) than in the control plants (78.18%).

The statistical analysis shows that the relative water content is highly significant in the leaves with all cadmium treatments.

Discussion

Effect of heavy metals on chlorophyll content of *Atriplex canescens*

- The accumulation of chlorophyll is related to the content of the medium of heavy metals culture.
- The chlorophyll content decreases in the presence of heavy metals and increases in its absence.
- Chlorophyll a accumulates less by contribution to chlorophyll b, for both controls and stressed plants. A decrease in the chlorophyll content is observed in the plants subjected to metallic stress.

Effect of lead on chlorophyll content of *Atriplex canescens*

The results obtained show a significant regression as a function of the lead dose present in the medium. Cenkci *et al*., (2010) have shown that exposure to lead plants can result to inhibition of photosynthesis, due to deformation of chloroplast structures (Stefanov *et al*., 1995), and to reduction of photosynthetic yield, and to a limitation of the rate CO_2 assimilation (Singh *et al.,* 2010), a stomatal closure (Parys *et al.,*1998). Lead may also inhibit certain enzymes involved in the Calvin cycle (Vallee and Ulmer, 1972). This inhibition is due to the decrease in the synthesis of chlorophyll, plastoquinone, and carotenoid (Burzynski, 1987), an altered electron transport (Rashid *et al*., 1994).

Effect of cadmium on chlorophyll content of *Atriplex canescens*

The results of our study showed that the chlorophyll content decreases with the increase in Cd concentration in the culture medium, which confirms the results of Singh et *al*., (2004) which report a frequent degeneration of the quantity of chlorophyll in plants exposed to different concentrations of heavy metals.

Cadmium may also have direct or indirect action on chlorophyll content (Singh *et al*., 2010) through metabolic disturbances (Vassilev *et al*., 1997) or inhibition of chlorophyll biosynthesis enzymes (Padmaja *et al*., 1990). The decrease in photosynthetic activity in the presence of cadmium may also be due to a decrease in the content of the aerial parts in other pigments such as β-carotene or xanthophylls (Larbi *et al*., 2002).

Cadmium also acts on the electron transport process during photosynthesis. Cadmium affects the components of photosystems II (PSII) by altering their structures and/or activities (Sigfridsson *et al*., 2004).

Thus, the decrease in photosynthesis may be due to inhibition of chlorophyll synthesis by the blocking of Mg, Mn and Fe ions (Gopal and Rizvi, 2008), or by the increase in chlorophyllase activity (Drazkiewicz, 1994), or the destruction of chloroplasts (Sharma and Dubey, 2005).

Similar results have shown that the chlorophyll pigment content is reduced under the effect of lead in radish (Sun *et al.,* 2010), maize, *Chlorella vulgaris* and *Pfaffia glomerata* (Gupta *et al.,* 2011) ; Cadmium in maize*, Pfaffia glomerata* and Radish (El-Beltagi *et al.,* 2010) This effect is based on the concentration and duration of treatment with heavy metals.

Effect of heavy metals on the water content of *Atriplex canescens*

Our results show that the water content decreases at the level of the leaves of the *Atriplex canescens* under metallic stress compared to the control plant.

Similar results were obtained by Barcelo and Poschenrieder, 1990 ; Pandolfini *et al.,* 1992) which showed that the decrease in water content of plant organs is often noted during metal stress.

In another study conducted by Barcelo and poschenrieder, (1990), the transport of water into the plant was often blocked by metal stress.

Effect of lead on the water content of *Atriplex canescens*

The results show that the lead stress applied to the plant leads to a decrease in the water content of the leaves at 2500, 5000, 7500, and 10000 ppm of lead relative to the control.

Transpiration and relative water content also showed a decrease in the case of excessive concentrations of Pb, Cu, and Zn in sunflowers (Kastori *et al.,* 1992). These results also indicate that ETM also influence the plant-water relationship by inhibiting root hair formation, reducing the ability of plants to explore soil for water and nutrient absorption. Another consequence of the presence of ETM is an increase in the resistance of the water flow in the root system. Indeed, the permeability of the membranes is reduced by an increase in suberization and lignification (Menon, 2006). Concerning the transfer of water to the aerial parts, it is limited by the reduction in the number and diameter of vascular bundles (Pal *et al.,* 2006).

Results obtained by Brunet (2008) show that there are few differences in the relative water content (RWC) of leguminous plants (*Lathyrus sativus* L.) treated with lead, other studies have shown a decrease in transpiration rate and water content in plants (Sharma and Dubey, 2005).

Effect of cadmium on the water content of *Atriplex canescens*

The results found for cadmium stressed plants at different concentrations (2500, 5000, 7500 and 10000 ppm), show a decrease in the water content in the leaves of *Atriplex canescens*. The lowest water content in the leaves is obtained at a dose of 10000 ppm.

Many studies have identified a significant disturbance of the water status of plants treated with Cd. An excess of Cd disrupts several physiological metabolisms in the plant such as water absorption, evapotranspiration or respiration (Wang *et al.,* 2008).

Some studies have also shown that the transport of water decreases two to four times depending on the species and the concentration of cadmium (Barcelo *et al*., 1988; Marchiol *et al*., 1996). Barcelo *et al*., (1988) considered that this decrease was due to the inhibition of the division and the elongation of the xylemic cells. These authors have assumed that this is a consequence of the disturbances of the hormonal balance disturbances caused by cadmium.

Conclusion

The increase in the concentration of heavy metals induces a reduction in photosynthesis and inhibits the synthesis of chlorophyll, all these observed changes could be taken as biomarkers of toxicity of heavy metals on the Plant.

The relative water content and water deficiency in the leaves represent excellent indicators for estimating the state of hydration of plants against abiotic stresses. The effect of metal stress results in a decrease in the water content of the *Atriplex canescens*, depending on the increasing concentration of applied heavy metals.

At the end of the results found, we can conclude that the effect of metal stress in the *Atriplex canescens* reflects the slowing of the photosynthetic activity of the plants in the presence of cadmium and lead. This is accompanied by a reduction in stomatal conductance resulting from the closure of the stomatal. This phenomenon, coupled with the change in root structure, can result to changes in the water fluxes within the plant, disrupting the transfer of nutrients to the plant and thus its development on polluted soils.

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